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Prognostic value of local invasion patterns in upper gastrointestinal cancers

PhD thesis

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List of abbreviations

EAC: esophageal adenocarcinoma
ESQCC: esophageal squamous cell carcinoma
GERD: gastroesophageal reflux disease
GC: gastric cancer
GAC: gastric adenocarcinoma
GEJ: gastroesophageal junction
LNM: lymph node metastasis
LNR: lymph node ratio
TNM: Tumor-Node-Metastasis
EMR: endoscopic mucosal resection
ESD: endoscopic submucosal dissection
OS: overall survival
ICI: immune checkpoint inhibitor
TB: tumor bud
PDC: poorly differentiated cluster
EMT: epithelial-mesenchymal transition
ITBCC: International Tumor Budding Consensus Conference
CRC: colorectal cancer
HE: haematoxylin-eosin
CPS: combined positive score
DFS: disease-free survival
SARIFA: Stroma AReactive Invasion Front Area

1. Introduction

In the last few decades in Western societies, esophageal adenocarcinoma (EAC) has surpassed esophageal squamous cell cancer (ESQCC) in incidence and thus became the predominant cancer entity of the esophagus. According to the GLOBOCAN website, esophageal cancer was ranking in 22nd place in incidence and 13th place in mortality in Hungary in 2021. The shift in the proportions of ESQCC and EAC is also visible in Hungary, even though only 34.1% of all diagnosed esophageal cancers proved to be EACs, while 60% was ESQCC, while in Germany in 2020, 41% of all diagnosed esophageal cancers proved to be ESQCC and 51% was classified as EAC. (1,2) This phenomenon can be attributed to a change in risk factors, since the prevalence of smoking and alcohol consumption has decreased massively, while the prevalence of obesity and gastro-esophageal reflux disease (GERD) has increased. The former risk factors are also responsible for the development of gastric cancer (GC), which –although with decreasing incidence- is still the fifth most common malignancy and the fourth most common among cancers causing death worldwide. (1)

So far, both in esophageal, gastroesophageal junction (GEJ) and gastric cancers, the prognosis is based on the 8th edition of UICC/AJCC Tumor-Node-Metastasis (TNM) staging, which is comprised of the extent of the tumor and the presence of lymph node and distant metastases. The prediction of lymph node metastases (LNMs) is especially important for the choice of surgical approach, since early stage diseases in which lymphatic spreading of the tumor has not yet occured are manageable with endoscopic modalities, such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), while in more advanced cases with developed LNMs, gastrectomy/esophagectomy with lymph node dissection is preferred. (3,4)

The lymph node ratio (LNR) is an emerging but clinically not yet fully translated biomarker constisting of the number of metastatic lymph nodes divided by the absolute number of lymph nodes examined. (5) Currently, the evaluation of lymph node metastases according to the TNM system is based on the absolute number of LNMs in both esophageal and gastric cancers. (6,7) In GACs, the N stage is heavily influenced by the extent of the lymphadenectomy (D1 vs D2). Even in the case of a D1 lymphadenectomy, a minimum number of 16 lymph nodes is recommended for examination. However, in

many cases, this number is not reached, and even if it is, small numbers of examined lymph nodes can lead to a phenomenon called stage migration. LNR has been demonstrated to be an independent prognostic factor for overall survival (OS) in gastric as well as esophageal cancers. (5,8)

With personalized medicine on the rise, the subtyping of gastric and GEJ-cancers has come into a focus of interest, however, only a few markers have been incorporated into the routine diagnostics. (9) In the case of HER2 positive metastatic gastric- and GEJ-cancers, the HER2-inhibitor trastuzumab along with the use of the immune checkpoint inhibitor (ICI) pembrolizumab has been approved by the FDA, the latter drug no longer being tied to a PDL>1 combined positive score (CPS). (10) The assessment of the MSI-status by immunohistochemistry and PCR is also a strong predictor of response ICI therapy. However, the aforementioned methods require special equipment and trained personnel, while more cost-effective approaches would be ideal.

Recently, several novel prognostic factors determinable from routine haematoxylin-eosin stained histological sections have been identified as potentially useful markers in upper gastrointestinal tract tumors.

Tumor buds (TBs) and poorly differentiated clusters (PDCs) are manifestations of the same biological process, the epithelial-mesenchymal transition (EMT). During this process, malignantly transformed cells lose their epithelial features by ceasing the expression of certain cell adhesion molecules and the rearrangement of the cytoskeleton, while simultaneously gaining migratory potential by developing a mesenchymal phenotype. The distinction between TBs and PDCs lies in the cell cluster sizes, as TBs are comprised of up to four tumor cells, while PDCs are made up of more than five cells. However, only in two-dimensional sections do these clusters show up as individual cell groups, and according to the findings of Bronsert et al., in 3D spatial reconstruction of the tumor invasion, the invading tumor represents a branching pattern, with individual tumor cells detaching from the main tumor mass as the last step of the invasive process. TBs and PDCs are the cross-sections of these invasive branches, observable as cell clusters on a conventional histological slide. (11)

The assessment of TBs and poorly PDCs according to the recommendations of the International Tumor Budding Consensus Conference (ITBCC) has been incorporated in the UICC guidelines in the routine evaluation of colorectal cancers (CRCs) in 2017. Their

assessment can be performed on routine haematoxylin-eosin (HE) slides, they are quick to evaluate, increasing cost-and-time-effectiveness. (12) In CRCs, tumor budding is an independent adverse prognostic factor. In endoscopically treated patients with pT1 CRC, high degrees of tumor budding in postoperative histological samples indicate the higher possibility of LNMs, thus surgical resection has to be taken into consideration. In stage II CRCs, high tumor budding predicts shorter disease-free survival (DFS), and the patient may benefit from adjuvant therapy. (12)

Since then, several studies have examined the potential usefulness of TBs and PDCs in upper gastrointestinal (GI) tract cancers, more specifically in GCs and esophageal cancers (13–16), the focus being on the predictive quality of lymphatic metastasis formation and survival. So far, the assessment of TBs and PDCs has not been universally adapted into the routine diagnostics in neither of the aforementioned tumors, further studies containing higher numbers of cases are required for validation.

A further recently identified prognostic factor is the Stromal AReactive Invasion Front Areas (SARIFA) which is a histological phenomenon where tumor cells come into direct contact with adipocytes, without the presence of interposing stromal or inflammatory cells. During the process of tumor progression, the growing number of tumor cells and the consequential increased tumor metabolism results in hypoxia, which causes the cancer cells to shift their metabolism from glycolysis towards b-oxidation, increasing the utilization of free fatty acids by coming to direct contact with adipocytes. (17) While this entity has only been found and described recently, it has been proven that SARIFA is an independent adverse prognostic factor in CRCs and gastric cancers. (18,19) Although it seems promising, further confirmation is required to adapt it into the diagnostics of upper GI cancers. (15,19–23)

2. Aims and Objectives

1. We aimed to assess the relationship between the phenomenon of Stroma AReactive Invasion Front Area (SARIFA) and tumor budding (tumor buds, TBs and poorly differentiated clusters, PDCs) in esophageal cancers, as well as their relationship to the established clinicopathological factors.
2. We aimed to examine the prognostic role of SARIFA in squamous cell carcinomas, prevously only studied on adenocarcinomas, by assessing SARIFA on ESQCCs beside EACs,
3. We aimed to identify histologic markers that are able to predict LNMs in esophageal squamous cell cancers (ESQCCs) and esophageal adenocarcinomas (EACs) by the analysis of the invasion front.
4. We aimed to assess the prognostic impact of TBs, PDCs and SARIFAs on the overall survival of ESQCC and EAC patients.
5. We aimed to evaluate whether the tumor bud (TB)- and poorly differentiated cluster (PDC)-count assessed according to the protocols of the International Tumor Budding Consensus Conference (ITBCC) described in colorectal cancers is able to predict the occurence of lymph node metastases (LNMs) in gastric adenocarcinomas (GACs).

3. Materials and Methods

3.1. Case selection – Esophageal cancer

This study was designed to retrospectively investigate the cases of patients who underwent esophageal resection for esophageal squamous cell carcinoma (ESQCC) or esophageal adenocarcinoma (EAC) between 2008 and 2021, with the histopathological work-up of the tumor having been performed in the Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Budapest, Hungary. This study excluded patients with incomplete clinical data, those who experienced perioperative death within 30 days of surgery, and those who achieved complete remission after neoadjuvant therapy due to the entire disappearance of the tumor. As a result, 100 cases were included for analysis. Key clinicopathological characteristics of the cohort are presented in Table 1.

3.2. Case selection – Gastric cancer

The gastric cancer cohort consisted of all gastric cancer (GC) patients treated with primary total or partial gastrectomy from the institutional archive of the Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Budapest, Hungary, between 2008 and 2018. Diagnostic glass slides were available for analysis in 374 cases of gastric adenocarcinoma (GAC). In this study, patients with incomplete clinical data, patients who received neoadjuvant therapy (this is a major difference to the esophageal cohort) were excluded from the study, whereas patients who died in the perioperative period were excluded only from survival analyses but were included in other assessments. A total of 290 patients were part of the study, the cohortclinicopathological characteristics are presented in Table 5.

3.3. Clinical and pathological data

Regarding both patient cohorts, the detailed clinical histories of the patients were collected from the electronic patient register of Semmelweis University (MedSol software). Pathological data were gathered from the register of the Department (MedRec software), while additional follow-up data were obtained from the National Cancer Registry (National Institute of Oncology, Budapest, Hungary).

3.4. Histological work-up and assessment

The diagnosis of ESQCCs and EACs and the assessment of the pTNM stage was based on the 8th edition of the UICC guidelines. (24) The impact of neoadjuvant therapy was evaluated using the Mandard score (25), which indicates the remaining tumor tissue in post-neoadjuvant histological samples. Patients were categorized as responders (Mandard scores of 2, 3, and 4) or non-responders (Mandard score of 5), with the cases showing complete tumor remission after neoadjuvant therapy (Mandard score of 1) were excluded from this study. Regarding the GC cohort, the adenocarcinoma subtypes were defined according to the Lauren classification (26) and the pTNM stage was determined following the 8th edition of the UICC/AJCC guidelines. (27) Multiple haematoxylin-eosin-stained (HE-) sections from each patient were analyzed using brightfield microscopy. Sections clearly representing the invasion fronts were scanned and digitized with a Panoramic 1000 Digital Slide Scanner (3D Histech, Budapest, Hungary). Tumor bud (TB) and poorly differentiated cluster (PDC) counts in both esophageal and gastric cancer cases, along with the occurrence of Stroma AReactive Invasion Front Area (SARIFA) in the esophageal cancer cases, were evaluated on the digital slides using the CaseViewer software (version 2.4.0, 3D Histech, Budapest, Hungary). Two specially trained investigators (A.J. and L.Z. for the esophageal cancer study; A.J. and L.Sz. for the gastric cancer study), blinded to clinical and outcome information, performed these evaluations. TBs and PDCs were assessed following the International Tumor Budding Consensus Conference (ITBCC) recommendations. (12) TBs were defined as either a single individual tumor cell or a solid, gland-like group of up to four tumor cells per the ITBCC guidelines. PDCs were identified as solid, gland-like nests containing five or more tumor cells. (12) TBs and PDCs were quantified in the invasive front of each tumor using the hot-spot method, within a 0.785 mm^2 area at a total magnification of 200×, a method established for evaluating tumor budding in colorectal cancer by the ITBCC guidelines. The TB and PDC counts recorded by the two investigators were averaged for further analysis. In cases with substantial discrepancies, the case was reassessed jointly by additional investigators with expertise in tumor histopathology (É.K. and G.L.) to reach a consensus. Based on the total number of TBs in the hot-spot area, cases were classified into groups based on budding grades (Bd 0: 0, Bd 1: 1–4, Bd 2: 5–9, and Bd 3: ≥10 TBs; Figure 1a–c). (12) PDCs were similarly categorized according to the number of cells

within each cluster, resulting in groups of PDCs containing 5–9 cells, 10–14 cells, and 15 or more cells. PDC grades were determined in a manner similar to TB grades, based on the total number of PDCs (independent of the number of cells in each cluster) within the analyzed hot-spot area (PDC 0: 0, PDC 1: 1–4, PDC 2: 5–9, and PDC 3: ≥10 PDCs; Figure 1d,e).

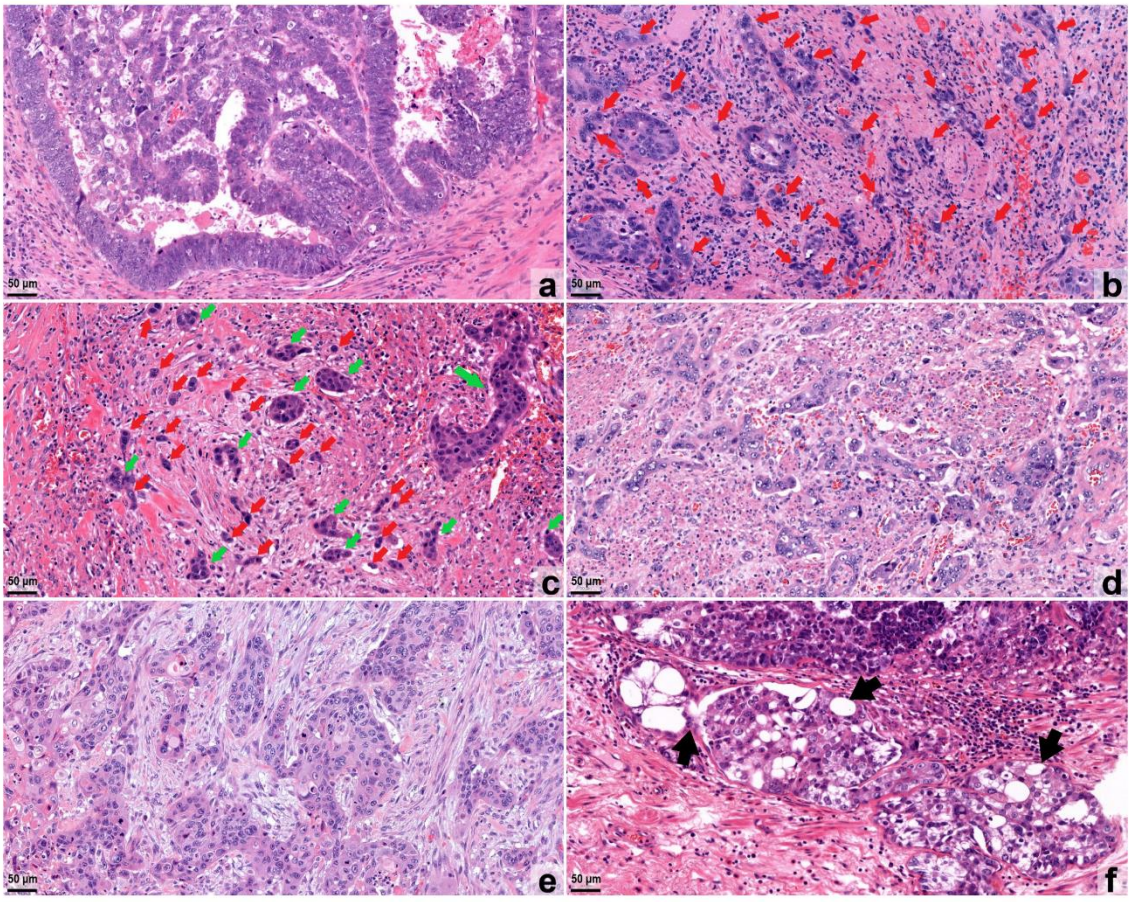

Figure 1. Examples of the histopathological appearance of tumor buds (TBs), poorly differentiated clusters (PDCs), and Stroma AReactive Invasion Front Areas (SARIFAs) in the investigated digital slides of hematoxylin–eosin (HE)-stained sections of esophageal squamous cell carcinoma (ESQCC) and esophageal adenocarcinoma (EAC). (**a**) "Pushing border"-type invasion pattern of an EAC with no visible TB or PDC (Bd 0/PDC 0). (**b**) EAC exhibiting high tumor budding (Bd 3, TBs are indicated by red arrows). (**c**) ESQCC case showing high tumor budding (Bd 3). (**d**) EAC of high PDC status (PDC 3, PDCs are indicated by green arrows). (**e**) Infiltration of an ESQCC with high PDC status (PDC 3). (**f**) Appearance of SARIFA (indicated by black arrows) in an EAC case. Original magnification: 200×. (Jakab et al. 2024.) (15)

SARIFA is an area of the invasion front in which a tumorous gland or a group of at least 5 tumor cells are in direct contact with adipocytes without stromal reaction. (19) SARIFA was assessed on the same slide as the TBs and PDCs, following the method described by Grosser et al.. (19) The presence of SARIFA anywhere on the slide (SARIFA+) or the absence of this (SARIFA−; Figure 1f) gives the base of the evaluation.

3.5. Ethical approval

The study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of Semmelweis University, Budapest (SE-RKEB 242-1/2021 for the esophageal cancer study and SE-RKEB 245/2019 for the gastric cancer study). Based on the current Hungarian law for scientific research, contacting the patients in order to obtain their informed consent is not required for retrospective studies. According to this, the Ethical Committee of Semmelweis University, Budapest, waived the informed consent procedure for this study. The study complies with the REMARK checklist of reporting recommendations for tumor marker prognostic studies.

3.6. Statistical analysis

All calculations and plotting were carried out in the R software environment (version 4.0.2) and R Studio portable (version 1.3.959). As in previous studies, the Bd 0, Bd 1, and Bd 2 groups were combined into the “TB low” category, while the Bd 3 group alone represented the “TB high” category for the calculations. PDC counts were grouped into “PDC low” (PDC 0, 1, and 2) and “PDC high” (PDC 3) categories in a similar manner. For the calculation of hazard/odds ratios in multivariable analysis, a two-tier reclassification of the variable categories was used in order to reach the most substantial dichotomous separation of the cases clinicopathologically (i.e., low/high grade), which provides greater statistical power and is a commonly used approach in clinical practice. Accordingly, not all available increments were used in the calculations, pT stages 1 and 2, as well as 3 and 4, were combined instead, resulting in a two-tiered scale (pT low vs. pT high). Similarly, the pN stages were combined into a two-tier system (pN0 vs. pN+). For the statistical analysis, continuous variables were expressed by their mean, range, and standard deviation (SD), while categorical variables were described as frequencies, with

their raw data having been plotted in 2 × 2 contingency tables and analyzed using Fisher's exact probability test. To identify the independent risk factors of lymph node metastases, univariable and multivariable logistic regression analysis with backward selection was applied. In the case of esophageal cancers, correlation of the number and size of independent tumor cell clusters and SARIFA status was carried out using the Mann–Whitney test. Survival analyses were performed using the Kaplan–Meier/log-rank method. The correlation between overall survival and the absolute number of TBs, PDCs, and SARIFA+/− statuses were analyzed using age-adjusted Cox proportional hazards regression with backwards stepwise selection. In the gastric cancer cohort, the relationship between the lymph-node ratio (LNR) and the Bd and PDC grade groups was examined pairwise by Wilcoxon's test, and the summarised groups by the Kruskal-Wallis test. In the gastric cancer study, patients who died within 30 days of surgery were not included in the survival analyses (perioperative death; n = 16/290; 5.52%), whereas in the esophageal cancer study, patients who died perioperatively were excluded from the study altogether. In multiple analyses the Benjamini-Hochberg method was applied to adjust the p value. All p-values were calculated as two-tailed, and were considered significant when $p < 0.05$.

4. Results

4.1. Cohort characteristics and differences in clinicopathological parameters of esophageal cancer cases

A total of 100 patient cases were selected for the study cohort, consisting of 80 males and 20 females. The mean age was 64.8 years (range 45–80). Histologically, the diagnosis was ESQCC in 57 cases and EAC in 43 cases. Despite LNMs being present in 47 cases, lymphovascular invasion was observable in only 24 cases. This matches the number of cases with observable perineural invasion. The mean lymph node ratio (LNR), the number of metastatic lymph nodes divided by the number of all examined lymph nodes per case was 0.15. 70 patients received neoadjuvant therapy (either chemo-, radio-, or chemo-radiotherapy), out of which 43 patients showed histological response. Based on our results, the age of EAC patients age was significantly higher than the age of ESQCC patients (mean age: 66.7 vs. 63.4 years, $p = 0.014$). Ultimately, **the presence of LNMs** (present vs. absent, 19 vs. 38 in ESQCC and 28 vs. 15 in EAC, $p = 0.023$), lymphovascular invasion (present vs. absent, 9 cases vs. 48 cases in ESQCC and 15 cases vs. 28 cases in EAC, $p = 0.0342$), perineural invasion (present vs. absent, 4 cases vs. 53 cases in ESQCC and 20 cases vs. 23 cases in EAC, $p \leq 0.0001$), LNR (0.10 in ESQCC and 0.23 in EAC, $p = 0.0080$), **and SARIFA** (present vs. absent, 19 cases vs. 38 cases in ESQCC and 27 cases vs. 16 cases in EAC, $p = 0.0046$) **was significantly higher in EACs than in ESQCCs**. However, regarding TB and PDC status, no significant difference could be observed between the two histological subtypes (Table 1).

Table 1. Cohort characteristics and comparison of clinicopathological parameters of ESQCC and EAC cases. Statistically significant results are highlighted in bold. Abbreviations: pT, pathological tumor (T) stage; pN, pathological lymph node (N) stage; pM, pathological distant metastasis (M) stage; LNR, lymph node ratio, the rate of tumorously involved lymph nodes; PDC, poorly differentiated cluster; SARIFA, Stroma AReactive Invasion Front Area. * Grade was determined only in cases where previous neoadjuvant therapy was not administered. ** Mandard score was determined only for patients who received previous neoadjuvant therapy (Jakab et al. 2024.) (15)

Cohort Characteristics		**Total**	**Squamous Cell Carcinoma**	**Adenocarcinoma**	***p***
Patients	n	100	57	43	-
Age	year	64.8	63.4 (45–80)	66.7 (42–80)	**0.0140**
Sex	male	80	42 (73.7%)	38 (88.4%)	0.0815
	female	20	15 (26.3%)	5 (11.6%)	
pT	low	41	28 (49.1%)	13 (30.2%)	0.0670
	high	59	29 (50.9%)	30 (69.8%)	
pN	absent	53	38 (66.7%)	15 (34.9%)	**0.0023**
	present	47	19 (33.3%)	28 (65.1%)	
pM	absent	97	56 (98.2%)	42 (97.7%)	1
	present	3	1 (1.8%)	1 (2.3%)	
Vascular invasion	absent	78	44 (77.2%)	34 (79.1%)	1
	present	22	13 (22.8%)	9 (20.9%)	
Lymphovascular invasion	absent	76	48 (84.2%)	28 (65.1%)	**0.0342**
	present	24	9 (15.8%)	15 (34.9%)	
Perineural invasion	absent	76	53 (93.0%)	23 (53.5%)	**<0.0001**
	present	24	4 (7.0%)	20 (46.5%)	
Resection margin	tumor-free	78	46 (80.7%)	32 (74.4%)	0.4746
	positive	22	11 (19.3%)	11 (25.6%)	

Cohort Characteristics		**Total**	**Squamous Cell Carcinoma**	**Adenocarcinoma**	***p***
LNR	mean	0.15	0.10	0.23	**0.0080**
Grade *	low	16	11 (64.7%)	5 (38.5%)	1
	high	14	6 (35.3%)	8 (61.5%)	
Necrosis	absent	57	29 (50.9%)	28 (65.1%)	0.2208
	present	43	28 (49.1%)	15 (34.9%)	
Neoadjuvant therapy	no	30	17 (29.8%)	13 (30.2%)	1
	yes	70	40 (70.2%)	30 (69.8%)	
Mandard score **	responder	43	26 (65.0%)	17 (56.7%)	0.6204
	non-responder	27	14 (35.0%)	13 (43.3%)	
Tumor budding	low	47	30 (52.6%)	17 (39.5%)	0.2278
	high	53	27 (47.4%)	26 (60.5%)	
PDC	low	31	16 (28.1%)	15 (34.9%)	0.5165
	high	69	41 (71.9%)	28 (65.1%)	
SARIFA	absent	54	38 (66.7%)	16 (37.2%)	**0.0046**
	present	46	19 (33.3%)	27 (62.8%)	

4.2. Relationship of the SARIFA status and the size of the tumor cell clusters in the invasion front of esophageal cancers

To assess the relationship of TBs/PDCs and SARIFA within the same tumor, the distribution of the number of tumor cell clusters (either TB or PDC, i.e., combining the two onto one spectrum) was plotted according to the number of constituting cells in SARIFA-positive and negative cases. **In those EACs where small clusters of cells** (TBs and PDCs of up to 14 cells) **occurred in large numbers, the presence of SARIFA was found to be significantly more frequent than in the subpopulation where small cell clusters occurred in small numbers** (clusters of 1–4 tumor cell(s): $p < 0.001$, 5–9 tumor cells: $p < 0.001$, 10–14 tumor cells: $p = 0.024$, and ≥15 tumor cells: $p = 0.332$). This correlation was not observable in ESQCC (Figure 2).

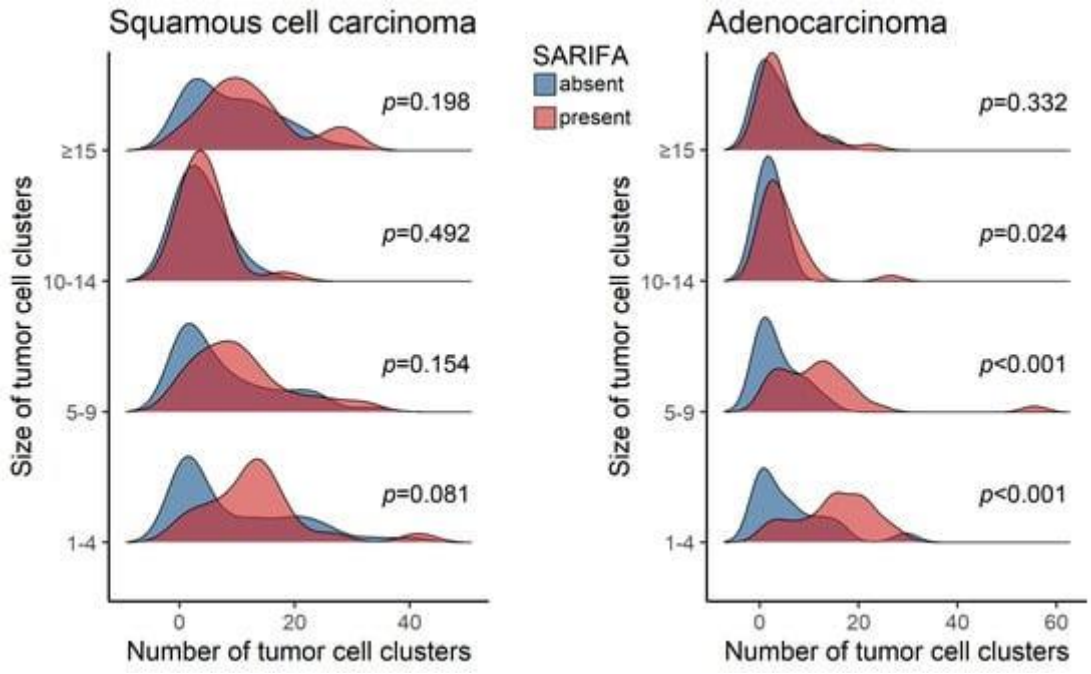

Figure 2. Tumor cell clusters of different sizes in the invasion front of SARIFA-positive and negative cases. Abbreviation: SARIFA—Stroma AReactive Invasion Front Area. (Jakab et al 2024.) (15)

4.3. Association between the TB/PDC/SARIFA status and the extent of the tumor in esophageal cancers

In EACs, high TB status and the presence of SARIFA were correlated with greater extent of the tumor ($p = 0.0162$ and $p = 0.0012$, respectively). However, the PDC status showed no significant correlation with the tumor extent. In the case of ESQCCs, no significant correlation could be observed (Table 2).

Table 2. Association between TB/PDC/SARIFA status and the extent of the tumor. Statistically significant results are highlighted in bold. Abbreviations: TB, tumor budding; PDC, poorly differentiated cluster; SARIFA, Stroma AReactive Invasion Front Area. (Jakab et al. 2024.) (15)

Squamous Cell Carcinoma		**Total**	**T-Low**	**T-High**	**p**
TB	low	26	12	14	0.7921
	high	31	16	15	
PDC	low	16	11	5	0.0819
	high	41	17	24	
SARIFA	absent	38	21	17	0.2630
	present	19	7	12	
Adenocarcinoma		**Total**	**T-Low**	**T-High**	**p**
TB	low	17	9	8	**0.0162**
	high	26	4	22	
PDC	low	15	6	9	0.3238
	high	28	7	21	
SARIFA	absent	16	10	6	**0.0012**
	present	27	3	24	

4.4. LNM prediction in esophageal cancers

The prognostic effect of TB low and high status, PDC low and high status and SARIFA-positive and negative status was assessed, respectively, on the development of LNMs in both ESQCC and EAC patients (Table 3). Univariable logistic regression showed a significant positive correlation between lymph node positive (pN+) status and the low/high TB category ($p = 0.0006$) and the presence of vascular invasion ($p = 0.0352$) in ESQCCs. The lymph node positive status was significantly associated with a higher T stage ($p = 0.0424$) and the high TB status ($p = 0.0006$) as revealed by the multivariable analysis; thus, besides stage, **TB is** also **an independent prognostic factor for LNMs in ESQCC**. In EACs, univariable regression showed that only the SARIFA positive status was associated with lymph node positivity ($p = 0.0054$). Multivariable analysis also revealed a significant positive association ($p = 0.0111$), thus confirming that **SARIFA positivity is an independent prognostic factor for LNM in EACs** (Table 3).

Table 3. Uni- and multivariable logistic regression analyses for elucidating the effect of TB/PDC/SARIFA status on the development of lymph node metastases in esophageal squamous cell cancers (A) and adenocarcinomas (B). Statistically significant results are highlighted in bold. Abbreviations: TB, tumor budding; PDC, poorly differentiated cluster; SARIFA, Stroma AReactive Invasion Front Area; n.s., not significant. (Jakab et al. 2024) (15)

(A)			
Parameter		**Squamous Cell Carcinoma**	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Age	years	1.007 (0.935–1.087) 0.8430	n.s.

(A)			
Parameter		**Squamous Cell Carcinoma**	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Sex	male/female	0.491 (0.0995–1.872) 0.3270	n.s.
TB	low/high	12.273 (3.291–61.133) ***0.0006***	25.32 (4.88–222.0) ***0.0006***
PDC	low/high	4.667 (1.103–32.334) 0.0609	n.s.
T stage	low/high	2.750 (0.876–9.390) 0.0909	6.035 (1.244–46.336) ***0.0424***
SARIFA	absent/present	1.083 (0.314–3.534) 0.8956	n.s.
Vascular invasion	absent/present	4.20 (1.123–16.941) ***0.0352***	n.s.
Lymphovascular invasion	absent/present	1.886 (0.414–8.198) 0.3931	n.s.

(A)

Parameter		Squamous Cell Carcinoma	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Perineural invasion	absent/present	7.40 (0.870–156.349) 0.0938	n.s.
Resection margin	tumor-free/ positive	0.886 (0.172–3.696) 0.8730	n.s.
Neoadjuvant therapy	no/yes	2.60 (0.697–12.694) 0.1839	n.s.

(B)

Parameter		Adenocarcinoma	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Age	years	1.025 (0.961–1.094) 0.4460	n.s.

(B)			
Parameter		**Adenocarcinoma**	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Sex	male/female	0.780 (0.115–6.486) 0.7988	n.s.
TB	low/high	2.413 (0.671–9.054) 0.1800	n.s.
PDC	low/high	2.188 (0.592–8.276) 0.2390	n.s.
T stage	low/high	3.208 (0.834–13.047) 0.0924	n.s.
SARIFA	absent/present	7.333 (1.903–32.532) ***0.0054***	11.0 (2.0–91.78) ***0.0111***
Vascular invasion	absent/present	5.600 (0.881–109.986) 0.1230	n.s.
Lymphovascular invasion	absent/present	362,730,000 (<0.0001-Infinity) 0.9940	n.s.

(B)			
Parameter		**Adenocarcinoma**	
		Univariable	**Multivariable**
		OR (CI) ***p***	**OR (CI)** ***p***
Perineural invasion	absent/present	3.667 (0.987–15.955) 0.0626	n.s.
Resection margin	tumor-free/ positive	3.079 (0.656–22.413) 0.1910	n.s.
Neoadjuvant therapy	no/yes	0.768 (0.175–2.989) 0.7100	n.s.

4.5. Analysis of the overall survival of esophageal cancer patients

The impact of TB low/high, PDC low/high, and SARIFA positive/negative status on the overall survival was analyzed by Kaplan–Meier curves and the log-rank test. No significant association was observed in ESQCCs, nor in EACs (Figure 3).

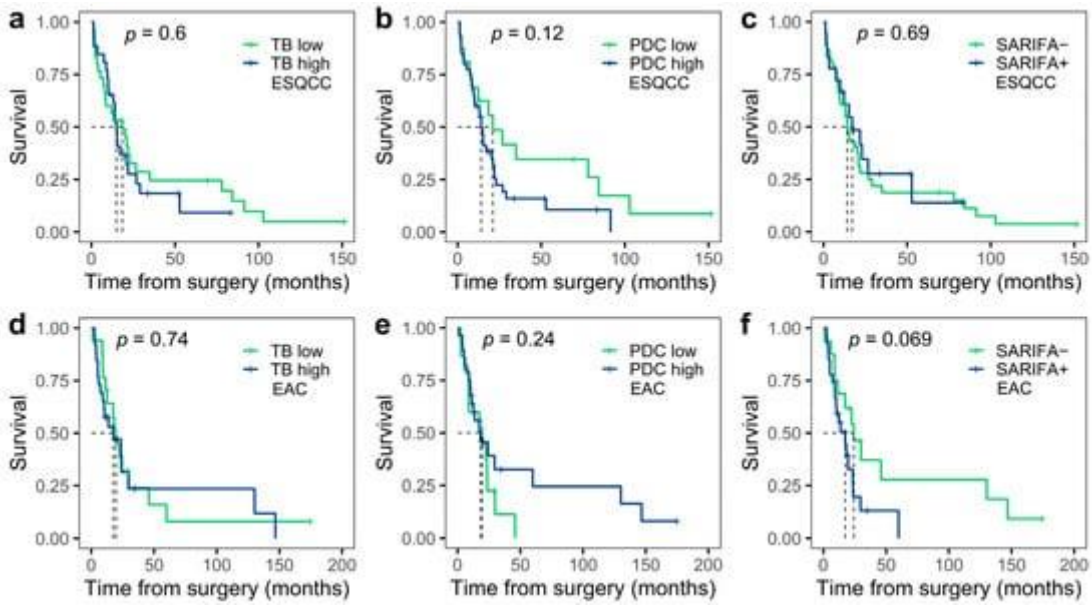

Figure 3. Kaplan–Meier curves depicting overall survival for subgroups with TB low/high, PDC low/high, and SARIFA+/− status. TB low/high in squamous cell carcinoma (**a**), PDC low/high in squamous cell carcinoma (**b**), SARIFA+/− in squamous cell carcinoma (**c**), TB low/high in adenocarcinoma (**d**), PDC low/high in adenocarcinoma (**e**), and SARIFA+/− in adenocarcinoma (**f**). The dashed lines in the figure show median survival. Abbreviations: TB, tumor budding; PDC, poorly differentiated cluster; SARIFA, Stroma AReactive Invasion Front Area; ESQCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma. (Jakab et al. 2024) (15)

For further investigation, an age-adjusted Cox regression model for OS was applied using the absolute numbers of TBs and PDCs counted in each case as continuous variables. **In ESQCCs**, **both TB** (p = 0.0269) **and PDC** (p = 0.0377) **showed a statistically significant negative association with overall survival**, but no such relationship was found with SARIFA. In EACs, neither of the aforementioned factors showed a significant

association, however, in the case of SARIFAs, a similar trend not reaching the threshold of significance was observable (p = 0.0658) (Table 4).

Table 4. Analysis of the relationship between overall survival and the absolute numbers of TBs and PDCs, as well as SARIFA status in an age-adjusted Cox proportional hazards regression model. Statistically significant results are highlighted in bold. Abbreviations: ESQCC, esophageal squamous cell carcinoma; PDC, poorly differentiated cluster; SARIFA, Stroma AReactive Invasion Front Area; EAC, esophageal adenocarcinoma. (Jakab et al. 2024) (15)

ESQCC	**OR**	**CI**	***p***
Tumor budding	1.036007	1.0040570–1.068972	**0.0269**
Age	0.994523	0.9533010–1.037527	0.7993
PDC	1.018223	1.0010303–1.035712	**0.0377**
Age	0.998127	0.9571506–1.040857	0.9301
SARIFA	0.872240	0.4520001–1.683178	0.6840
Age	1.001310	0.9599019–1.044505	0.9520
EAC	**OR**	**CI**	***p***
Tumor budding	1.008108	0.9628542–1.055489	0.7300
Age	1.029528	0.9921378–1.068327	0.1230
PDC	0.978292	0.9467089–1.010929	0.1900
Age	1.026867	0.9914605–1.063539	0.1390
SARIFA	2.098440	0.9527029–4.622074	**0.0658**
Age	1.032400	0.9929269–1.073441	0.1089

4.6. Characteristics of the gastric cancer cohort

A total of 290 patients were eligible for the study, with a gender distribution of 107 females and 183 males. The clinicopathological characteristics of the cohort are shown in Table 5. The mean age was 66.13 years (range 34–89). The surgical approach in 184 (63.45%) cases was total gastrectomy, while 106 (36.55%) patients underwent subtotal gastric resection. The tumor originated in the cardia in 98 cases (33.79%) and in the distal part of the stomach in 192 (66.21%) cases. According to the Lauren classification system (26) of GCs, a total of 159 (54.83%) cases of intestinal morphology, 107 (36.90%) cases of diffuse morphology and 27 (9.31%) cases of mixed morphology were identified. During the histological examination, a total of 222 cases showed tumor-free resection margins (R0; 76.55%), while in 68 cases the resection was found to be incomplete (R1; 23.45%). Pathological staging was following the 8th TNM classification (27)(ref), resulting in 8.97% (26 cases) of the patients being classified as pT1, 7.24% (21 cases) as pT2, 50.35% (146 cases) as pT3 and 33.45% (97 cases) as pT4. In six cases (2.07%), the tumor was graded as well differentiated (G1), 92 (31.72%) tumors were graded as moderately differentiated (G2) and 192 (66.21%) tumors as poorly-/undifferentiated (G3). Lymphovascular invasion was observable in 103 cases (35.52%) while perineural invasion was documented only in 81 cases (27.93%). The median of the follow-up time was 34 months (ranged 1–137 months). Grading according to the ITBCC guideline resulted in three patients (1.03%) being classified in Bd 0, 19 (6.55%) patients in Bd 1, 40 (13.79%) patients in Bd 2 and 228 (78.62%) patients in Bd 3 (Figure 5). Similarly, categorizing the patients by PDC grade, a total of one (0.35%) patient was classified as PDC 0, 16 (5.52%) patients as PDC 1, 39 (13.45%) patients as PDC 2 and 234 (80.69%) patients as PDC 3 (Figure 5).

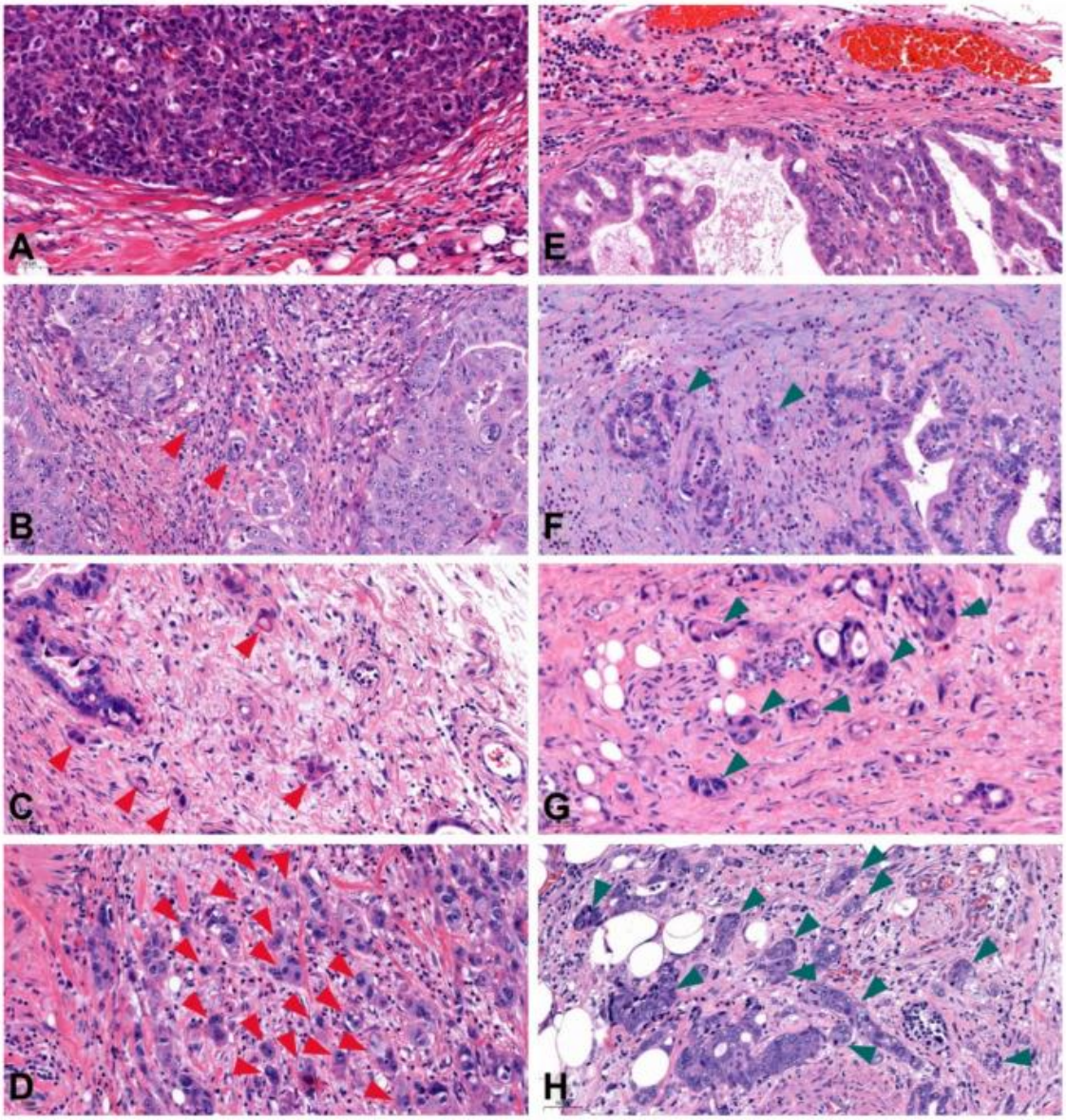

Figure 4. Tumor budding and PDC grades assessed according to ITBCC recommendations in gastric adenocarcinomas. Tumor buds are indicated by red arrowheads, PDCs by green arrowheads. (A) Bd grade 0 (no tumor bud in the hot spot), (B) Bd grade 1 (1–4 tumor bud/hot spot), (C) Bd grade 2 (5–9 tumor bud/hot spot), (D) Bd grade 3 (≥10 tumor bud/hot spot), (E) PDC grade 0 (no PDC in the hot spot), (F) PDC grade 1 (1–4 PDC/hot spot), (G) PDC grade 2 (5–9 PDC/hot spot), (H) PDC grade 3 (≥10 PDC/hot spot); (PDC: poorly differentiated cluster, original magnification 20×). (Szalai et al. 2022) (14)

Table 5. Clinicopathological characteristics of the gastric cancer cohort. (pT: Primary tumor extent, pN: Regional lymph node metastasis, LNR: lymph node ratio, UICC: Union for International Cancer Control, PDC: poorly differentiated cluster, pM: Distant metastasis). (Szalai et al. 2022.) (14)

Variable		***n*/%**
Number of patients	n	290
Age at surgery (years)	mean, range	66.12759 (34–89)
Sex	female	107
	male	183
Extent of gastrectomy	total	184
	subtotal	106
Location	cardia	98
	fundus–body–antrum	192
Lauren classification	intestinal	159
	diffuse	107
	mixed	27
Resection margin	R0	222
	R1	68
Perineural invasion	present	81
	absent	209
Lymphovascular invasion	present	179
	absent	111

Variable		***n*/%**
Tumor differentiation	well	6
	moderate	92
	poor	192
pT	Ia	12
	Ib	14
	II	21
	III	146
	IVa	73
	IVb	24
pN	0	80
	1	52
	2	56
	3a	62
	3b	40
LNR	mean, range	0.38 (0.0–1.0)
Distant metastasis (pM)	present	17
	absent	273
UICC Stage	IA	23
	IB	15
	IIA	35
	IIB	49

Variable		***n*/%**
	IIIA	52
	IIIB	57
	IIIC	42
	IV	17
Tumor budding grade	Bd 0	3
	Bd 1	19
	Bd 2	40
	Bd 3	228
PDC grade	PDC 0	1
	PDC 1	16
	PDC 2	39
	PDC 3	234
Follow-up period	mean, range	33.803 (1–137)
Death	n	228 (78.62%)
Perioperative death	n	16/290 (5.52%)

4.7. Association of TBs and PDCs with clinicopathological parameters in gastric cancers

The asscociations between clinicopathological parameters and the TB or PDC low and high categories were examined. **Tumor budding was positively correlated with** the tumor's Lauren-phenotype (*p*=0.0003), incomplete surgical resection (*p*=0.0174), presence of perineural and lymphovascular invasion (*p*=0.0295 and *p*=0.0032, respectively), pT status (*p*=<0.0001), grade (*p*=<0.0001), UICC stage (*p*=<0.0001) and **the presence of LNMs** (*p*=<0.0001). High PDC category showed a significant positive correlation with the Lauren-phenotype (*p*=0.0086), incomplete surgical resection (*p*=0.048), the presence of perineural and lymphovascular invasion (*p*=0.0417) and *p*=0.0481, respectively), pT status (*p*=<0.0001) and grade (*p*=<0.0001).

4.8. Effects of TBs/PDCs on LNMs of different gastric cancer subtypes

The presence of LNMs was assessed among patients with low and high TB as well as low and high PDC categories, respectively (Table 6). Univariable logistic regression showed a significant positive correlation between pN positive status and the low/high TB category (p<0.0001), pT stage (p<0.0001), Lauren phenotype (p<0.0008), grade (p<0.0001), positive resection margin (p=0.008) and the presence of lymphovascular or perineural invasion (p<0.0001 and p=0.016, respectively). Multivariable logistic regression analysis with backward stepwise selection yielded the **high TB grade** as **an independent predictor for LNMs in both the total cohort** (*p* = 0.019, OR: 2.86, CI: 1.197–6.877) **and in the intestinal type adenocarcinomas** (*p* = 0.038, OR: 2.778, CI: 2.78–7.52). A similar trend not reaching the threshold of significance was found between the PDC high grade and the presence of LNMs (total cohort: *p* = 0.057; intestinal cohort: *p* = 0.075) (Table 6).

Table 6. Prediction of lymph node metastases in gastric adenocarcinomas (pT: Primary tumor extent, pM: Distant metastasis, n.i.: not included in the analysis /as only intestinal type was analysed in these columns, Lauren type was not included as a variable here/, abbreviations: n.s., statistically non-significant; TB, tumor bud; PDC, poorly differentiated cluster; pM, distant metastasis) Statistically significant p values obtained from multivariable analyses are displayed in bold). (Szalai et al. 2022) (14)

Para-meter		**Total Cohort**		**Intestinal Type**	
		Univariable	**Multi-variable**	**Univariable**	**Multi-variable**
		OR (95% CI) ***p***	**OR (95% CI)** ***p***	**OR (95% CI)** ***p***	**OR (95% CI)** ***p***
TB	low/ high	3.65 (2.03–6.62) <0.0001	**2.86 (1.20–6.88)** ***0.019***	2.36 (1.18–4.76) 0.015	**2.78 (1.07–7.52)** ***0.038***
PDC	low/ high	1.61 (0.86–2.98) 0.132	**0.37 (0.13–0.99)** ***0.057***	0.94 (0.44–1.94) 0.860	n.s. 0.075
Sex	M/F	1.12 (0.66–1.93) 0.68	n.s. n.s.	1.25 (0.62–2.60) 0.534	n.s. n.s.
Age	years	0.98 (0.95–1.00) 0.051	n.s. n.s.	0.96 (0.92–0.99) 0.009	**0.96 (0.92–0.99)** ***0.036***
pT	I–II/ III–IV	14.80 (7.20–32.65) <0.0001	**7.19 (3.09–17.75)** ***<0.0001***	7.58 (3.24–19.49) <0.0001	**4.34 (1.65–12.32)** ***0.004***

Para-meter		Total Cohort		Intestinal Type	
		Univariable	**Multi-variable**	**Univariable**	**Multi-variable**
		OR (95% CI) ***p***	**OR (95% CI)** ***p***	**OR (95% CI)** ***p***	**OR (95% CI)** ***p***
pM	pM0/ pM1	6.52 (1.30–118.59) 0.071	n.s. n.s.	3.81 (0.63–72.85) 0.221	n.s. n.s.
Lauren-type	intestinal / other	2.23 (1.42–3.63) 0.0008	n.s. n.s.	n.i. -	n.i. -
Grade	G1/ G2/ G3	3.23 (1.99–5.35) <0.0001	n.s. n.s.	2.02 (1.13–3.69) 0.019	n.s. n.s.
Residual tumor	absent/ present	2.67 (1.34–5.83) 0.008	n.s. n.s.	1.53 (0.66–3.75) 0.334	n.s. n.s.
Lympho-vascular invasion	absent/ present	11.32 (6.24–21.42) <0.0001	**6.09 (3.10–12.30)** ***<0.0001***	7.56 (3.70–16.23) <0.0001	**5.11 (2.30–12.02)** ***<0.0001***
Peri-neural invasion	absent/ present	2.21 (1.19–4.35) 0.016	n.s. n.s.	3.11 (1.37–7.75) 0.009	n.s. n.s.

Furthermore, the metastatic involvement of lymph nodes in patients of different Bd and PDC grades was also assessed. The ratio of tumorous lymph nodes to total lymph nodes excised **(LNR) was significantly correlated with 0 + 1/2/3 Bd grades in both the total cohort** ($p < 0.0001$) **as well as in intestinal and diffuse subtypes of GCs** ($p = 0.012$ in intestinal cancers and $p = 0.03$ in diffuse cancers, respectively). Grading for **PDC showed a significant correlation with LNR only in the total cohort** ($p = 0.02$) **and in diffuse GCs** ($p = 0.011$) (Figure 6).

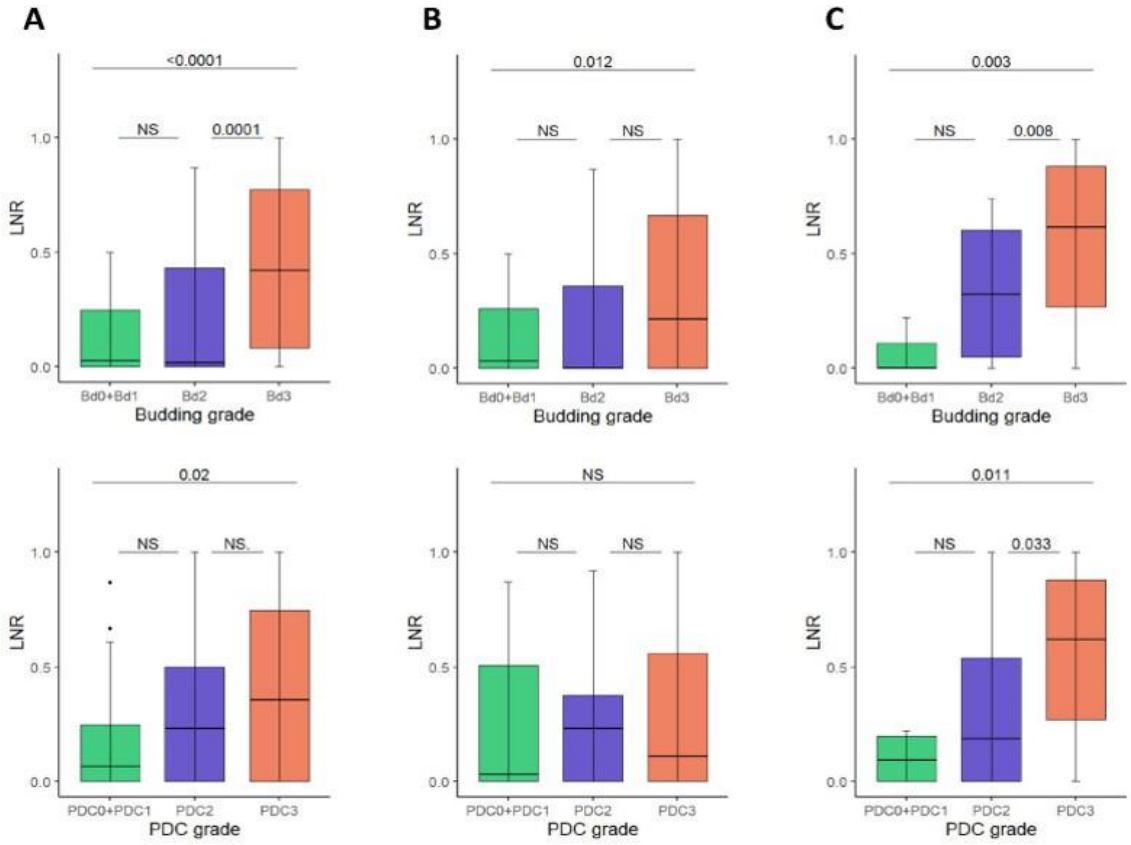

Figure 5. Association of the the lymph node ratio (LNR) and tumor budding/PDC grades. (A) Total cohort, (B) Intestinal type gastric cancers, (C) Diffuse type gastric cancers. PDC: poorly differentiated clusters. (Szalai et al. 2022.) (14)

5. Discussion

There is an increasing need for biomarkers that are able to predict lymph node metastases (LNMs), thus aiding the personalized choice of therapeutic approaches. Tumor budding and poorly differentiated clusters (PDC) are established prognostic factors in the diagnostics of colorectal cancers (CRCs). The evaluation of the aforementioned phenomena are quick and cost-effective, since they are performed on conventional haematoxylin-eosin (HE)-slides under a few minutes. Their applicablity in upper gastrointestinal (GI) tract tumors has been in the focus of many recent studies. (28–30)

In our own studies, we have assessed tumor buds (TBs) and PDCs in a cohort of gastric adenocarcinomas (GACs) consisting of intestinal, diffuse and mixed GACs and in a separate cohort of esophageal cancers, containing both esophageal squamous cell cancers (ESQCCs) and esophageal adenocarcinomas (EACs). In addition, our study group performed the evaluation of the newly discovered biomarker Stroma AReactive Invasion Front Area (SARIFA) in the latter cohort, with a special emphasis on their predictive role regarding the development of LNMs.

Our cohort of ESQCCs provides unique opportunity to assess TB, PDC and SARIFA in this histological subtype of esophageal cancer, which has shown a drastic decline in incidence in favor of EACs in the Western world in the last decades. (1) To our knowledge, our study group was the first to assess the presence of SARIFA in squamous cell cancers. We found that SARIFA was significantly less frequent in ESQCCs than in EACs ($p=0.0046$) and its prognostic role was not significant (compared to EAC) for either survival or LNM prediction). Hopefully, more studies will be conducted in the future that elucidate the role of SARIFA in the squamous cell cancers of other organs.

As of yet, the relationship between TB, PDC and SARIFA has been scarcely studied, although it is clear that invading cell clusters have more potential to directly reach the adipocytes located in the deeper layers of organ walls. The findings of the study conducted by Ulase et al. support this theory, as they found a weak positive correlation between SARIFA and the clusters of tumor budding in a large cohort consisting of gastric and gastroesophageal adenocarcinomas. (31) This is also supported by the results of Martin et al., the study group that originally described SARIFA, as they also found a weak positive correlation between the number of tumor budding clusters (TBs and PDCs) and

the presence of SARIFA in CRCs. (18) In our own study on esophageal cancers, the correlation between TBs and SARIFA did not prove to be significant, however, we found that combining the TB-and PDC cell groups onto one spectrum, SARIFA occurs significantly more frequently in tumors where small cell clusters (TBs and PDCs of less than 15 cells) occur in greater numbers. This migh suggest that the arbitrary stratification of cell clusters based on the number of constituting cells does not accurately represent their biological properties. As of yet, the cut-off values of TBs and PDCs are based on studies conducted on CRCs, further studies containing larger numbers of esophageal cancers are required. (12,29,32)

Regarding the differences in the lymphatic dissemination of EACs and ESQCCs, in our cohort we found that EACs have a significantly higher rate of LNMs ($p=0.0023$), lymphovascular ($p=0.0342$) and perineural invasion ($p<0.0001$) and higher lymph node ratio (LNR) ($p=0.0080$) than ESQCCs. Our results conflict with the findings of Stein et al., as they found that LNMs occur more frequently in early stage ESQCCs than in EACs. (33) However, the underlying mechanism of lymphatic tumor spread might offer a possible explanation to this difference, as the chronic inflammation, characteristic of the precursor states of EAC, causes damage to the superficial lymphatic system of the esophagus, but not effecting the deeper, submucosal lymphatic vessels. Our cohort contained twice as many advanced stage EACs than early stage diseases, whereas in the case of ESQCCs, the number of early and advanced stage cancers were near equal. The findings of Lagarde et al. also support our results, since they found a significant positive correlation between higher pT stages and the higher frequency of lymphovascular invasion and LNMs in their cohort, which contained over three times more EACs than ESQCCs. (34)

Previous studies suggested the prognostic efficiency of tumor budding and SARIFA is influenced by the extent of the tumor in GI adenocarcinomas. (19) In our own study's EAC subcohort a higher T-stage was positively correlated with high TB score ($p=0.01621$) and the presence of SARIFA ($p=0.001232$), while in ESQCCs, no such correlation could be observed. This correlation can be explained in the case of SARIFA by the higher number of adipocytes in the adventitial layer, so that more advanced (T3-T4) tumors are more likely to encounter adipocytes. However, it is important to note that adipocytes are also present in smaller numbers in the submucosal layer of the esophageal

wall and as perivascular adipocytes in the muscularis propria layer. However, even this relationship is not clear, e.g. Grosser et al. found significant differences between the SARIFA positivity of pT1-2 vs pT3-4 cases in their study of different cohorts of GAC patients. The external validation cohort (TUM-cohort) had statistically significantly more SARIFA+ tumors among the pT3-4 cancers, whereas the TCGA-STAD cohort had an equal distribution of SARIFA+ tumors between the pT1-2 and pT3-4 categories. (21)

Moreover, SARIFA proved to be an independent prognostic factor regarding the development of LNMS in EACs (p=0.011), while the TB and PDC score were not able to accurately predict the aforementioned event.

In ESQCCs, TB proved to be an independent prognostic factor for the development of LNMs, while it was not able to predict LNMs in EACs. This is an interesting contrast to our findings regarding GACs, where tumor budding had prognostic significance, as the TB score was predictive of the LNMs in both the total cohort as well as the intestinal and the diffuse subcohort. This suggests that gastric/GEJ adenocarcinomas and EACs have different metastatic behaviour, in which the biological role of tumor budding may also differ. The predictive role of tumor budding in GACs would indicate the potential usefulness of tumor budding in the prognostic evaluation of EACs as well, however, in our own cohort, high TB was not predictive of the development of LNMs in EACs. This contrasts with the findings of Landau et al., who were able to prove the prognostic power of TB on the development of LNMs in a study conducted on a large cohort of early stage EACs containing 210 cases. (35) On the other side, in our study, SARIFA was proved to be an independent prognostic factor for the development of LNMs in EACs.

Furthermore, higher LNR was positively correlated with a higher degree of tumor budding in the total gastric cancer cohort and the intestinal as well as the diffuse subcohort, while the same correlation was observable only in the total and the diffuse subcohort regarding PDCs. However, the PDC score was not predictive of LNMs in esophageal nor in gastric cancers, although a trend not reaching the threshold of significance was observable in both cohorts.

Besides LNMs, the prediction of the overall survival (OS) is also a key step in reaching the optimal therapeutic stratification. Despite the TB and PDC grade categories (directly taken from the ITBCC guidelines for CRC evaluation) not being predictive of the OS in ESQCCs, the absolute number of invasive cell clusters in the TB and PDC categories

proved effective in predicting the OS in this histological subtype. This finding sheds light on the need for the revision of the ITBCC criteria regarding the cut-off value of TB and PDC scores for each tumor type specifically, before implementing them in the routine diagnostics of ESQCC or in other cancer entities. Lymph node ratio (LNR) has been proved to be an independent prognostic factor regarding OS in GACs and esophageal cancers. (5,8,36,37) In our study conducted on GACs, high LNR positively correlated with a high TB and a high PDC score. No such correlation was found in our esophageal cancer cohort, however, LNR was significantly higher in EACs than in ESQCCs.

During the analysis of the OS of EAC patients, SARIFA showed a trend towards prognostic significance without reaching it, which might be attributed to the small number of cases constituting the EAC subcohort. Moreover, our esophageal cancer cohort contained far fewer adenocarcinomas than our gastric cancer cohort, and in comparison to GACs, the proportion of low-stage cancers among EACs was even lower. The fact that SARIFA has not been shown to be prognostic with respect to OS could possibly be explained by the context that, with the progression of the tumor, the prognostic role of histological invasive patterns lessen and other prognostic factors become more important in the prediction of OS. As well as, the patients' general fitness and factors that are more directly related to the resilience against cancer-related systemic symptoms, paraneoplastic diseases and the side effects of the therapeutic regime might be more important in the survival prediction of patients suffering from advanced stage diseases. Hopefully, further studies containing larger numbers of EAC cases will prove the usefulness of SARIFA in the prediction of OS besides LNMs.

6. Conclusions

1. Tumor budding is an independent predictor of the development of lymph node metastases (LNMs) in esophageal squamous cell carcinomas (ESQCCs), while in esophageal adenocarcinomas (EACs) LNMs are predicted by the presence of Stroma AReactive Invasion Front Area (SARIFA).
2. Our study group was the first to assess the phenomenon of SARIFA on squamous cell cancers, previously only studied on gastrointestinal adenocarcinomas. We found that SARIFA occurs significantly less frequently in ESQCCs than in EACs.
3. Examining the associations of invasive patterns and clinicopathological factors in EACs, we found that SARIFA positivity is associated with a greater number of small cell clusters on the invasion front (TBs and PDCs up to 14 tumor cells) and a higher extent of the tumor (T3 and T4), the latter also being associated with a higher degree of tumor budding. No such correlation was found in ESQCCs.
4. Results of the survival analyses revealed that while the TB and PDC grades are not predictive of overall survival (OS), the absolute number of cell clusters in the categories of TBs and PDCs proved to be an independent prognostic factor of the OS of ESQCC patients. Therefore, the ITBCC criteria derived from colorectal cancers need to be re-evaluated and adapted to esophageal cancers before incorporating the assesment of tumor budding into the routine diagnostic protocols.
5. In the total gastric cancer (GC) cohort and the intestinal GC subcohort, the lymph node ratio (LNR) showed a significant positive correlation with both the TB and PDC grade, while in diffuse GCs, LNR was correlated only with the PDC grade. Moreover, based on the multivariable analysis, TB grade proved to be an independent prognostic factor for LNM development in intestinal GCs, outperforming the PDC grade in this manner.

7. Summary

Local invasion patterns are useful biomarkers determinable from conventional histologic slides. Tumor budding and poorly differentiated clusters (PDCs) are histologic manifestations of the epithelial-mesenchymal transition. They have been incorporated into the routine diagnostics of colorectal cancers in 2016, when the International Tumor Budding Consensus Conference (ITBCC) has established the standard methods of their evaluation. Stroma AReactive Invasion Front Area (SARIFA) is a newly discovered phenomenon, where tumor cells make direct contact with adipocytes. We have assessed tumor buds (TBs) and PDCs in a cohort of gastric adenocarcinoma patients, and in a separate cohort of esophageal cancer patients with the addition of SARIFA. Both cohorts comprised of distinct histological subtypes. The gastric cancer (GC) cohort contained intestinal and diffuse adenocarcinomas, while the esophageal cancer cohort was constituted of squamous cell cancers (ESQCCs) and adenocarcinomas (EACs). We collected and digitized the histologic slides of the patients along with obtaining their clinical and pathological data, yielding 290 cases in the gastric cohort and 100 cases in the esophageal cohort available for analysis. Our results proved that TBs, PDCs and SARIFA have strong correlations with other established clinicopathological biomarkers, as well as with each other. Each have distinct advantages, tumor budding is an independent prognostic factor of lymph node metastases (LNMs) in ESQCCs, while SARIFA predicts the same consequence in EACs. We have found that SARIFA occurs more frequently in EACs containing greater numbers of small cell clusters (TBs and PDCs of less than 15 cells), while also being characteristic of higher T stage (3 and 4) EACs. Moreover, we also assessed SARIFA in ESQCCs, which to our knowledge is the first occasion of evaluating the aforementioned marker in squamous cell cancers. In gastric cancers, although TBs outperformed PDCs in predicting the development of LNMs, both TBs and PDCs correlate with the lymph node ratio. Our results revealed that TB and PDC scores are not predictive of the overall survival of esophageal cancer patients, however, the absolute number of cell clusters in the TB and PDC categories are an independent prognostic factor for the aforementioned endpoint, shedding light on the need to revise the cut-off value before implementing TBs and PDCs in the routine diagnostics of esophageal cancers.

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